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## THE RELATION OF THE OPSONIC INDEX TO THE LEUCOPENIA AND LEUCOCYTOSIS FOLLOWING INJECTIONS OF HEATED BACTERIAL CULTURES.\*†

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IN the course of a series of experiments on rabbits on the effect of injections of killed bacteria on the leucocytes of the circulating blood, it was found, as pointed out by others, that an initial leucopenia was produced which was followed by a marked leucocytosis. It occurred to us that possibly some relation existed between the leucopenia and the negative phase, and the leucocytosis and the positive phase, of the opsonic index. If such a relation should prove constant, it might be of some practical as well as theoretical significance. There seemed to be a slight possibility of substituting simple leucocyte counts for the cumbersome opsonic technique as a means of determining the reaction of the individual to the injection of dead bacteria, and with this idea in mind our animal experiments were continued.

Metchnikoff<sup>1</sup> and his pupils found that intraperitoneal and intra-venous injections of a culture of cholera vibrios were followed by an immediate diminution in the number of the leucocytes. Howard<sup>2</sup> showed: (1) that intraperitoneal injections of cold salt solution into guinea-pigs caused a slight leucocytosis in four or five hours; (2) that intraperitoneal and subcutaneous injections of typhoid toxins and intraocular injections of killed typhoid bacilli produced a very rapidly developing and marked primary hypoleucocytosis which was followed by a marked hyperleucocytosis.

Staubli<sup>3</sup> made intraperitoneal injections of typhoid toxin and noted a primary hypoleucocytosis followed by a considerable hyperleucocytosis. Dean<sup>4</sup> found that after injection of diphtheria toxin

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<sup>1</sup> Kolle and Wasserman, *Handbuch*, 4, p. 380.

<sup>2</sup> *Jour. Med. Res.*, 1907, 17, p. 237.

<sup>3</sup> *Deut. Arch. f. klin. Med.*, 1905, 85, p. 286.

<sup>4</sup> *Jour. Path. and Bact.*, 1908, 12, p. 154.

there was an immediate and continuous rise in the number of the leucocytes of the circulating blood except when a neutralizing dose of antitoxin was given at the time of, or shortly before, the injection of the toxin. Marshall<sup>1</sup> found that intravenous injection of large doses of killed staphylococci caused a slight primary rise, followed in 25 hours by a fall, in the opsonic index; but by dilution experiments the phagocytosis was found to fall quickly to a minimum.

#### TECHNIQUE OF THESE EXPERIMENTS.

In these experiments healthy medium-sized rabbits were used. The injected material consisted of from one to six 24-hour agar slants of *B. coli* or *B. pyocyaneus* suspended in physiological salt solution and heated to 70° C. for two hours. Fifteen rabbits were used. Eight were injected with *B. pyocyaneus*, six with *B. coli*, and one with cold salt solution. Blood counts were made and blood collected for the opsonic determination immediately before, and at intervals of 20 minutes to four hours after, the injection for the first day, and once or twice daily thereafter. The usual care was observed in collecting the blood.

We are inclined to agree with Bunting<sup>2</sup> that rabbits are satisfactory animals for experimental blood work. He found that "counts made on successive days have shown only the slightest variations, either in the total count or in the differential." In our experiments an average of 18 counts on normal rabbits, taken without reference to time of day or feeding, gave 8,500 leucocytes per cu. mm. By far the greatest number ranged between 6,000 and 9,000.

The usual technique was employed in estimating the opsonic content of the serum. In the case of *B. pyocyaneus*, however, a special difficulty was encountered. After incubating a mixture of the serum, leucocytes, and suspension of these bacteria, it was almost impossible to find an intact leucocyte in the stained smear. The cytoplasm showed marked "digestion," and in certain instances only the nuclei, markedly disintegrated and indistinctly stained, could be found.

In 1899, Gheorghiewsky<sup>3</sup> noted that in 15 to 20 minutes after

<sup>1</sup> *Jour. Path. and Bact.*, 1908, 12, p. 378.

<sup>2</sup> *Jour. Exp. Med.*, 1908, 8, p. 629.

<sup>3</sup> *Ann de l'Inst. Pasteur*, 1889, 13, p. 298.

intraperitoneal injection of suspensions of *B. pyocyaneus*, the leucocytes obtained from the peritoneal cavity had lost their motility, had become round and swollen, and showed abnormal staining of their chromatin. *In vitro*, he found degeneration of the leucocytes when mixed with a suspension of *B. pyocyaneus*. In order to prevent this "digestive" action on the leucocytes in our mixtures it was necessary to suspend the organisms in physiological salt solution, centrifuge them for an hour, pipette off supernatent fluid, resuspend them in salt solution, and again centrifuge them for a few minutes to get rid of clumps. Normal rabbit serum contains a strong agglutinin for *B. pyocyaneus* which also interferes with opsonic technique. This difficulty could be obviated only by diluting the sera.

#### RESULTS.

Of the fifteen rabbits used, five died. Two of these had been given *B. coli* and three *B. pyocyaneus*. Four died within five hours after the injection, the fifth died on the second day. The four dying quickly showed practically the same condition; that is, a very prompt and pronounced fall in both leucocytes and opsonins. Forty-five minutes after intraperitoneal injection of three 24-hour glycerine agar slants of *B. pyocyaneus* which had been heated to 66° C. for two hours, the number of leucocytes had dropped from 5,800 to 1,100 per cu. mm. At the same time, the phagocytic index fell from 2.3 to 0.15. There was a very slight fluctuation about these low figures until death occurred in convulsions four hours after injection (Chart 1). In the case of a rabbit injected with four 14-hour glycerine agar slants of *B. coli* heated to 66° C. for two hours, in one hour and 20 minutes there was a fall in leucocytes from 7,600 to 1,800 and in the opsonic index from 0.9 to 0.25. Death occurred in convulsions two hours and 25 minutes after injection without any noteworthy change in the leucocytes or opsonins (Chart 2).

The animal which lived until the second day differed somewhat in its reaction. It received four 12-hour glycerine agar slants of *B. pyocyaneus* killed at 66° C. There was a prompt fall in the number of leucocytes from 4,000 to 800, after which the number increased gradually. The opsonic index at the same time rose from 0.6 to 1.1 and remained at about this point for the remainder of the day.

Shortly before death the leucocytes had risen to 3,000 and the opsonic index had fallen to 0.5.

In the case of the rabbits which survived, whether subcutaneous or intraperitoneal injections were given or either organism used, the results were practically identical. In every case there was a prompt fall in the number of the leucocytes. The lowest point was reached from three to seven hours after injection. The diminution in number varied from 2,000 to 7,700 leucocytes per cu. mm., the average

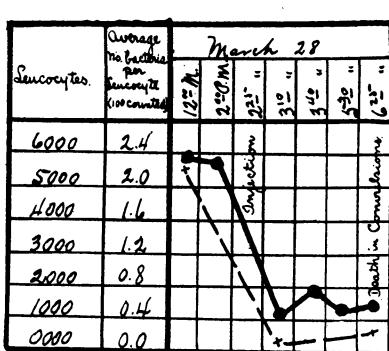


CHART 1.—Solid line=Leucocytes. Broken line=Opsonic Index. Intraperitoneal injection of three agar slants. *B. pyocyaneus*. Killed at 66° C.

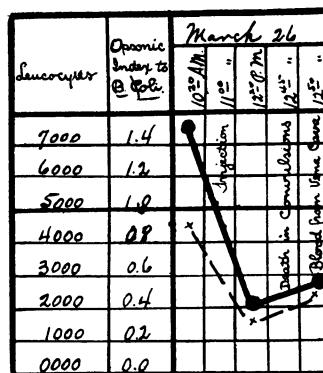


CHART 2.—Solid line=Leucocytes. Broken line=Opsonic Index. Intraperitoneal injection of four 14-hour agar slants. *B. coli*. Killed at 66° C.

fall being 4,800. On reinoculation the lowest point was reached in from one-half to two hours.

During the first two to eight hours after injection the opsonic index showed little variation; in some instances there was even a slight rise. After this a rather gradual decline took place, the lowest point being reached on the second to the fourth day. The negative phase of the opsonic index is thus seen to really begin about the time, or very shortly before, the period of leucopenia ends (Charts 3, 4, and 5).

On the second to the fourth days, usually the second and third, after injection, the leucocytes reached their highest point. In one case the count reached 37,000 per cu. mm. The increase in the number of leucocytes varied from 1,500 to 31,000 per cu. mm. above the count made previous to inoculation. On the third or fourth day the leucocytes dropped rather suddenly to normal and remained

practically within normal limits. In a few instances (Chart 3) a secondary leucopenia occurred, as mentioned by Howard.

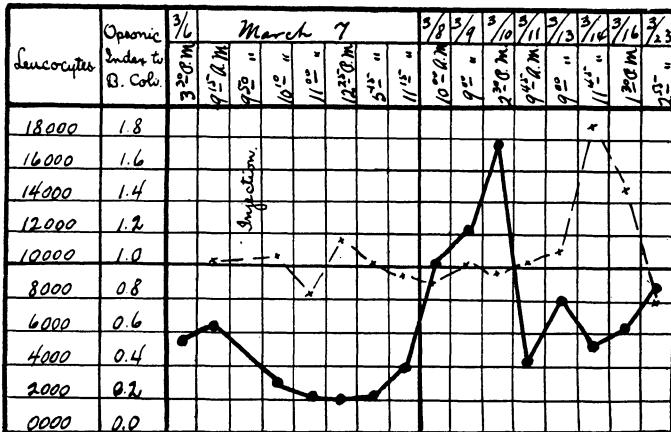


CHART 3.—Solid line=Leucocytes. Broken line=Opsonic Index. Intraperitoneal injection of three agar slants. *B. coli*. Killed at 66° C.

The opsonic index rose more gradually and reached its maximum on the fourth to the eighth days after the first injection, and on the

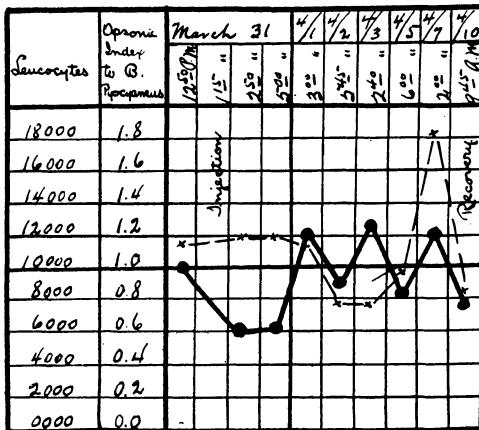


CHART 4.—Solid line=Leucocytes. Broken line=Opsonic Index. Subcutaneous injection of three agar slants. *B. pyocyaneus*. Killed at 66° C.

fourth to the tenth days after the second injection. An average of all the estimates made showed the highest indices on the sixth day. In the course of 10 to 12 days the opsonic index returned to normal

and sometimes remained below normal for two or three days. Thus it is seen that the positive phase of the opsonic index bears practically the same relation to the period of leucocytosis that the negative phase bears to that of leucopenia. The opsonic reaction to the injection is of slower development and of longer duration than the leucocytic reaction. Not infrequently the period of greatest leucocytosis was coincident with that of the lowest opsonic indices.

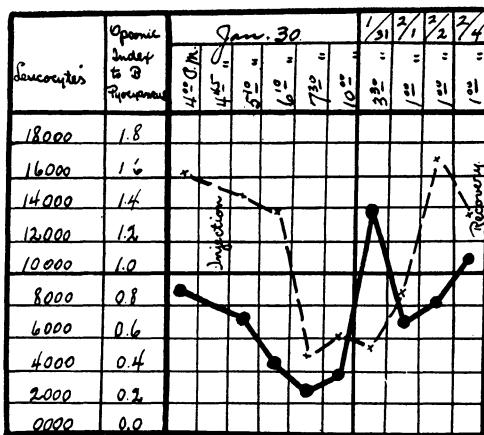


CHART 5.—Solid line=Leucocytes. Broken line=Opsonic Index. Intraperitoneal injection of four agar slants. *B. pyocyaneus*. Killed at 66° C.

Special attention may be called to two practical deductions from these experiments. First, the conditions found in the animals that died suddenly suggest the possibility that in so-called foudroyant infections in man, the sudden overwhelming of the human organism is rendered possible only by a complete paralysis of its entire protective mechanism. In all four of these animals both leucocytes and opsonins fell to a most abnormally low point and showed no signs of reacting. It does not seem unreasonable to suppose that the lysins and other protective bodies in the serum underwent similar changes. In all the animals that recovered, some of which received larger doses than the ones that died, the opsonins held their own until the leucocytes had begun to increase in number. This idea is further borne out by the results of injection of live bacteria (see Chart 6). When both leucocytes and opsonins fell quickly and showed no tendency to react,

the animal died quickly; but when the opsonins showed little or no change until the number of leucocytes began to increase, the animal usually recovered, or at least lived several days.

Second, intraperitoneal injections have a more powerful action on the leucocytes than do subcutaneous injections. (Compare Charts 3 and 4.) This is probably due to the more rapid absorption

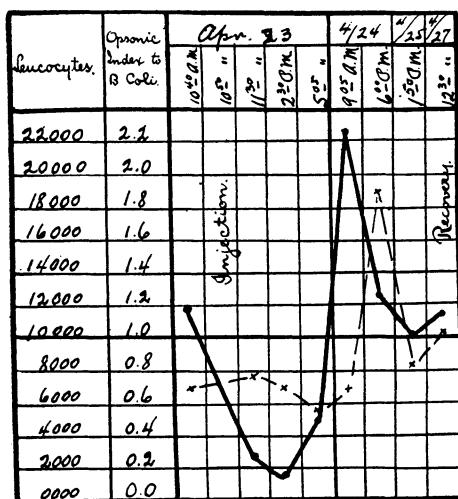


CHART 6.—Solid line=Leucocytes. Broken line=Opsonic Index. Intraperitoneal injection of one agar slant of living *B. coli*.

of the injected material from the peritoneal cavity. On the other hand, as already pointed out by one of us,<sup>1</sup> subcutaneous injections have an equally, if not more, favorable action in stimulating the production of opsonins.

#### SUMMARY.

Briefly summarized, then, the injection of heated bacterial cultures causes, in from 20 minutes to four hours, a leucopenia, followed, in two or three days, by a marked leucocytosis. The leucocytes usually return to the normal in four days. The opsonic index shows little or no variation for the first few hours after injections. The negative phase then sets in and lasts from two to four days. After this there

<sup>1</sup> *Jour. Infect. Dis.*, 1907, 4, p. 595.

is a gradual rise, the maximum being reached on the fourth to the eighth days. A gradual fall to normal occurs between the sixth and twelfth days. The leucocytes, therefore, are evidently more sensitive to bacterial injections than the opsonic index. Their variations are greater, the period of greatest leucopenia precedes the greatest fall in the opsonins, and the period of greatest leucocytosis precedes the greatest rise in the opsonic index, which usually reaches its maximum only after the leucocytes have returned to normal.